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Review / *Derleme*

Aethina tumida (Small Hive Beetle; SHB) and *Tropilaelaps* spp. Mite; an emerging threat to Turkey Honey Bees

Levent AYDIN

Bursa Uludağ Üniversitesi, Veteriner Fakültesi, Parazitoloji Anabilim Dalı, Bursa, TÜRKİYE
ORCID: 0000-0002-2875-8003

✉Corresponding author: laydin@uludag.edu.tr
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Abstract: Turkey is the second largest honey producer in the world. However, the beekeeping sector, especially the export of honey and honey products, is not supported by income compatible with this asset. The main reasons for this discrepancy are the presence of honey bee diseases (predators) and their residues in honey. Due to the observation of *Aethina tumida* (small hive beetle; SHB) in Europe (Italy) and *Tropilealaps* spp. Iran's border regions close to Turkey, the Ministry of Agriculture and Forestry of the Republic of Turkey has raised its alert level for these two notifiable diseases. *Aethina tumida* is a fruit insect originally from South Africa and it has later adapted to bee colonies. In the early 2000s, the agent came to North America with imported fruits and caused serious bee losses. SHB, which has spread to 12 countries so far, caused serious economic losses in the countries it entered and could not be eliminated. *Tropilaelaps* spp. is an ectoparasite that cannot survive for long without honey bee brood it feeds on and needs to survive. *Tropilaelaps* mite even suppresses *Varroa* when found in a colony and extinguishes the colony in a short time. It is a fact that these two pests may spread rapidly in Turkey due to the practice of migratory beekeeping and they can cause serious damage to our beekeeping.
Keywords: *Aethina tumida*, honey bee, *Tropilaelaps* spp., Turkey.

Aethina tumida (Küçük Kovan Kurdu; KKK) ve *Tropilaelaps* spp. Akarı; Türkiye Bal Arıları için yakın bir tehdit

Özet: Türkiye, dünyanın en büyük ikinci bal üreticisidir; ancak arıcılık sektörü, özellikle bal ve bal ürünleri ihracatı bu varlıkla uyumlu gelirle desteklenmemektedir. Bunun başlıca nedeni bal arısı hastalık- zararlıları ve balda kalıntı bulunmasıdır. Son yıllarda, (Küçük kovan böceği; SHB) Avrupa'da (İtalya) ve *Tropilaelaps* spp. İran'ın Türkiye sınırında görülmesi nedeniyle bu iki bal arısı zararlısına karşı ilgi artmıştır. Türkiye Cumhuriyeti Tarım ve Orman Bakanlığı bu iki zararlıyı ihbarı zorunlu hastalıklar arasına almıştır. *Aethina tumida*, aslen Güney Afrika'dan gelen ve daha sonra arı kolonilerine adapte olmuş bir meyve böceğidir. 2000'li yılların başında ithal meyvelerle Kuzey Amerika'ya gelmiş ve ciddi arı kayıplarına neden olmuştur. Bugüne kadar 12 ülkeye yayılan SHB, girdiği ülkelerde ciddi ekonomik zararlara yol açmış ve eradike edilememiştir. *Tropilaelaps*, beslendiği ve yaşaması için gereken bal arısı kuluçkaları olmadan uzun süre yaşayamayan bir ektoparazittir. *Tropilaelaps* akarı, bir kolonide bulunduğunda *Varroa*'yı bile baskılar. Koloniyi kısa sürede söndürür. Ülkemizde gezici arıcılık uygulaması nedeniyle bu iki zararlının hızla yayılacağı ve arıcılığımıza ciddi zararlar vereceği bir gerçektir.
Anahtar sözcükler: *Aethina tumida*, bal arısı, *Tropilaelaps* spp., Türkiye.

Introduction

According to the statistics of the United Nations Food and Agriculture Organization (FAO) for 2019, there are approximately 90 million honey bee colonies all over the world. The highest numbers of colonies are found in Asia (48.4%), followed by Africa (19.3%), Europe (18.0%), America (12.9%) and Oceania (1.5%) (16). Considering TURKSTAT's 2020 data (17, 35), Turkey ranks third in the world in terms of the number of colonies (approximately eight million in total) and second in honey production (104,000 tons per year). Beekeeping activities are performed in 81 provinces of Turkey (36) by 82 662 registered professionals whose main source of income or side income is beekeeping, as well as families engaged in

hobby beekeeping. The most important risks of beekeeping in our country are breeding queen deficiency, bee diseases and pests, residue problems, and marketing (17, 36).

The occurrence of some diseases and predators in honey bees not only threatens the future of the colony and the apiary but also closely concerns human health (6, 16). As with all mammals, many factors threaten bees together with environmental conditions. Various arthropods such as *Varroa*, wax moth and *Tropilaelaps* play an important role in the transmission of various disease agents to colonies. Especially in recent years, global climate change and international trade pose an increasing threat to honey bee health. *Varroa* spp is the best example of many bee diseases, especially after the 1970s, the spread of other new bee pests all over the world (16).

In recent years, *Aethina tumida* (small hive beetle (SHB); South African origin) and *Tropilaelaps* spp. (Southeast Asian origin) spread rapidly around the world, SHB reaching Italy and *Tropilaelaps* western Iran. Both predators are known to be more pathogenic than *Varroa* spp. (4, 5, 7-9, 28, 29). One of the most important problems of beekeeping in our country is bee diseases and pests. Uncontrolled migratory beekeeping causes these diseases and pests to spread rapidly throughout the country in particular. Parasitic agents are at the forefront of these diseases and pests due to the damage they cause and the need for drug use-residue problems (7, 8, 10, 12, 13, 16).

Aethina tumida (Small Hive Bettle)

Small hive beetle, commonly known as *A. tumida*, is an insect located in the south of the Great Sahara in the African continent and is a close relative and/or one of the insects that attack plants in the form of strawberries, melons, and saplings and feed on debris. For this reason, it is estimated to be a fruit pest (7, 8, 12, 13).

Significance of the Disease: It was first described by Lundie in 1940 and detected in beehives in South Africa (24). It affects detrimentally honeycombs and weak colonies stored in the African continent, while strong colonies can deal with this disease. *A. tumida*, thought to have entered the United States through fruit and vegetable imports, was first identified in Florida in May 1998. Later, it was noticed in 20 regions within 2 years and spread to 7 states (18). Due to both the climate characteristics and the absence of natural enemies, it has become more harmful in America than the African continent and has reached a level that threatens beekeeping in this continent (26-28, 31-34). So far, the estimated economic loss has been around $200 million, and the insect has reportedly caused the extinction of about 20,000 colonies (27, 28). It should be kept in mind that epidemics in the United States may create major problems in the future, as these regions are similar to Turkey when the climatic characteristics of these regions are considered, and it should not be forgotten that it would be a very rational behavior to take precautions for this (31, 35).

Etiology:

Phylum: Artropoda
Subphylum: Antennata
Class: Insecta
Order: Coleoptera
Family: Nitidulidae
Genus: *Aethina*
Species: *Aethina tumida* (Murray) (30).

Adult *A. tumida* is 5-7 mm long, and has three pairs of legs, two pairs of wings, and a dark brown almost black color. The bees cannot sting these pests due to the strong chitin layer covering their body. The dorsal side of their body is covered with hair and spikes and they have strong wings (Figure 1A, B). They are able to fly up to 24 km, thus they can spread rapidly in the environment. Parasite eggs are fusiform and they are laid irregularly into the deep corners of the hives, which are hard to reach, and cells (Figure 1C). Its larvae are long, oval, and approximately 11 mm long, and have a whitish-light brown color (Figure 1D). Despite the larvae looking like a wax moth, they have three not well-developed legs and well-developed dorsal spikes, which distinguish them from the wax moth. The larvae feed on pollen and honey. They defecate in the cells (18, 20, 31).

Biology: *A. tumida* is brown and about the size of a mature insect. Matures emerging from the pupa attack the colonies again. Although both larvae and adults of *A. tumida* feed on honey and pollen, they also consume bee eggs from time to time. They do not only need beehives to stay alive and lay eggs; these insects also live in some fruits, especially melons, and they can lay their eggs on these fruits. Studies have shown that adults of *A. tumida* survive for up to two weeks without food and water. This development, which is in four periods, is completed in 38-82 days (Figure 2). Temperate climatic regions contribute to the formation of five generations per year. It can contribute to deflating of the hive as it can lay a large number of eggs (7, 8, 10, 18, 20, 31).

Distribution: Although *A. tumida* is known mainly as a fruit pest, it was first seen in beehives in South Africa in 1940 (18, 24). Seasonal conditions are crucial in the ecology of the parasite, and it is reported the existence of the pest in regions close to the tropical climate. Factors such as beekeeping, fruit growing and humidity in South Africa have greatly affected the development and breeding of *A. tumida* and increased its prevalence. The phenotypic characteristics of bees are important against this pest, and it poses a serious threat to bee colonies that are not weak and vicious (31).

Figure 1. *Aethina tumida* Adult A, B (dorsal and ventral), C Eggs and D Larva (Buss and Ratikan, University of Florida).

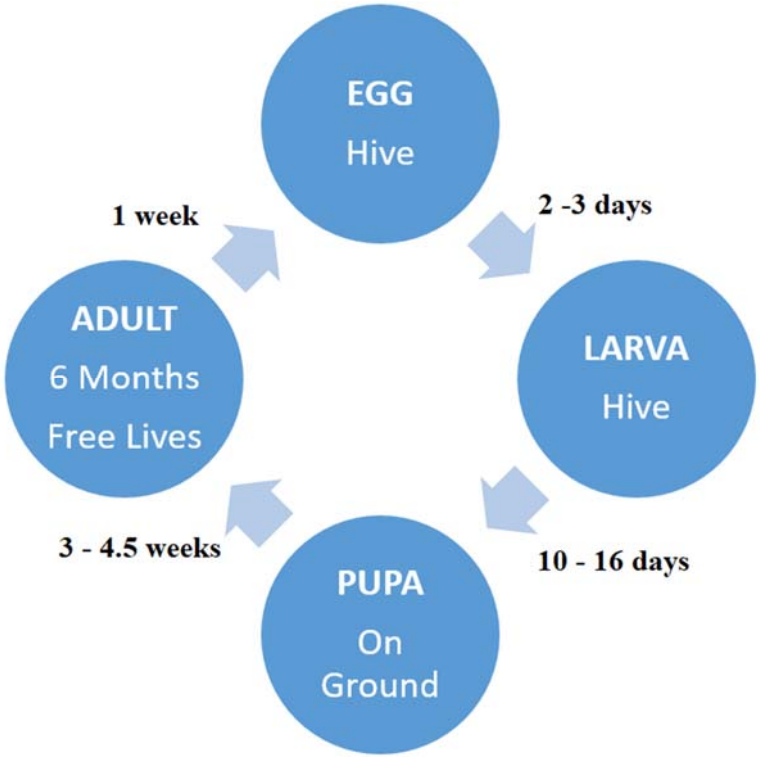

Figure 2. Biology in the ideal environment (Original).

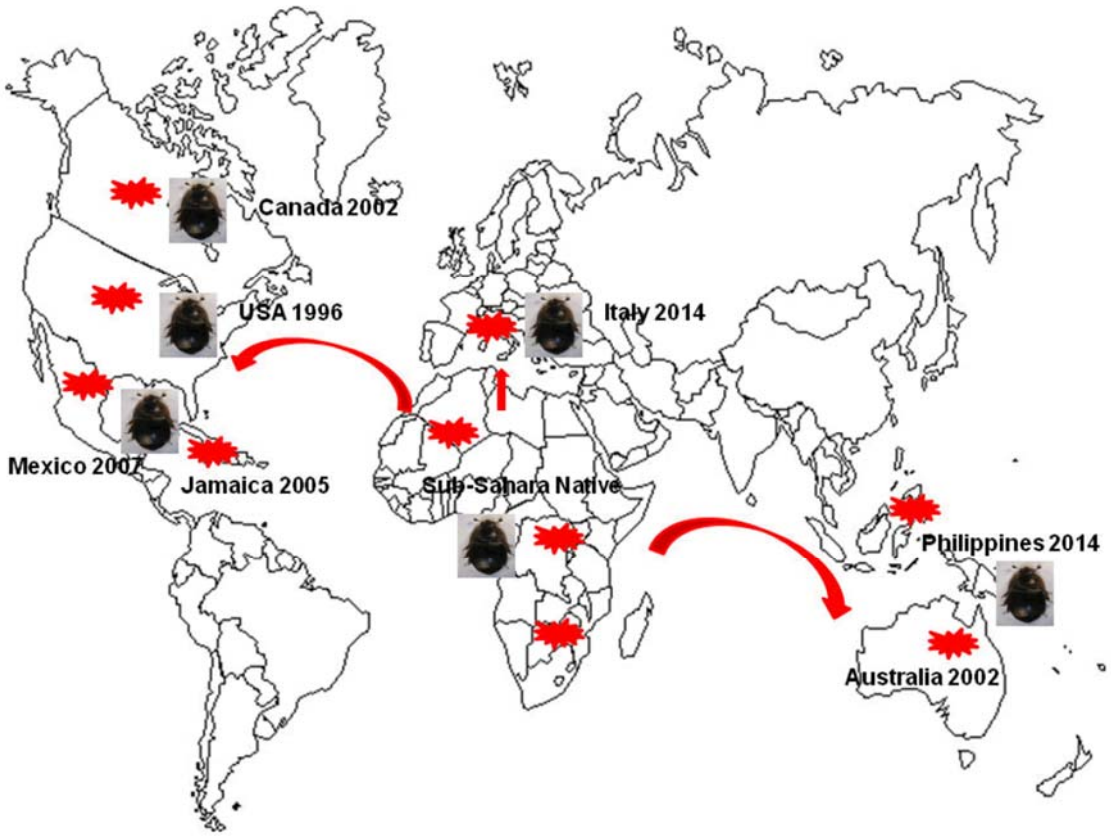

Figure 3. Prevalence of SHB (19).

According to the OIE, *A. tumida* has been recorded in North and Central America (USA, Canada, Mexico, Nicaragua, El Salvador), Cuba, Australia and southern Italy (5). There is no reliable information about its presence in Africa, but this pest is endemic in this region (7, 8, 10). Although this disease is not officially reported to the OIE, the OIE provides information on the presence of this pest in Egypt (2000) and Portugal (2004) in the Land Animal Health Code (5, 7). Finally, combined genetics data and beeswax trade data (FAO) for 12 confirmed small hive beetle (SHB) invasions (USA, Mexico, Jamaica, Cuba, Canada, Brazil, Costa Rica, USA [Hawaii], South Korea, Australia, Portugal and Italy) (Figure 3) (10). SHB has detected that the maximum entropy algorithm (Maxent Analysis) can damage the temperature factors in Southern Europe and North Africa in near future (21).

Clinical Symptoms: Due to their natural enemies and the hygienic behavior (aggressive) of African bees (*Apis mellifera scutellata*), great losses did not occur in the African continent and *A. tumida* population was kept under control. However, temperate countries (such as the USA) where it has been newly infected have become the primary pest (18, 20).

Pollution of honey and the use of pesticides in the control cause economic losses, and both the continuation of the hive and the vegetative production through pollination are endangered due to the weakening or extinction of the colony (37).

In the colonies of *A. tumida*, honeycombs are destroyed, bee larvae and eggs are consumed by the insect, a large number of larvae and eggs of *A. tumida* are found in the comb cells and a sour rotten orange (citrus) smell is felt as a result of fermentation due to the excrement they produce. Bees leave the honeycombs with brood and honey. Even when honey is left out of the hive, it is not consumed by other bees and insects (7, 10, 18, 31, 32).

Diagnosis: The diagnosis of infestation is made by identifying the larval and adult forms of the agent, as well as its eggs, which are much smaller (two-thirds) and few in number than bee eggs. Especially perception of the typical odor is suggestive of the disease and 15×15 cm honeycomb traps (which will remain for at least 3 days) can be used to catch the agent. If there are small hive beetles in a colony, they can be easily seen while the beekeeper is cleaning the hive. Insects are generally insusceptible to light conditions and will quickly seek shelter. Therefore, beekeepers can have an idea about the number of insects seen in the colony by checking the top of the hive. Once many insects are present at the top of the hive, brood chambers should be scrutinized to reveal the total insect population (7, 10, 20, 32, 34). To demonstrate

potential field use in recent years, the laboratory has tested sensitive loop-mediated isothermal amplification (LAMP) assay test program for rapid identification of *A. tumida* in honey bee hive ground debris and from crude extracts of samples or partial samples. In *A. tumida*, it is sensitive to an existing real-time polymerase chain reaction (PCR) and can be used worldwide as a useful biosafety tool for rapid detection of SHB (23, 32).

Control: Despite the use of many chemicals, complete success has not yet been achieved against *A. tumida*. However, strip applications of coumaphos have yielded relatively positive results. For protection, care should be taken to place the hives at least 20-30 cm above the ground with a forward inclination of 4°C. When combs with small hive beetles were kept at -12 °C for 24 hours or at -20 °C for 6-8 hours, it was observed that all developmental stages disappeared (28). The most assuring way to detect the presence of SHB is to use cardboard placed at the bottom of the hive. This method has been used successfully in the USA. This method is based on the SBH's tendency to search for dark habitats and holes and use them for hiding (10, 18, 20, 28).

Pesticide application containing 40% permethrin (40% EC) against larvae and pupae in the soil provides successful results. Spraying should be implemented late in the evening when the bees returned to the hive. The prepared medicine is applied to the area 45-60 cm wide in front of each hive. The application is repeated at 30-day intervals (18, 20, 31, 37).

Intra-hive drug control is one of the most dangerous and undesirable methods because of the risk of drug contamination to honey. Traps used to capture and destroy SHB in honey bee colonies are effective to reduce the contamination level (7, 10, 18, 22, 28). In addition, adult insects are susceptible to this fungus and three other general entomopathogenic fungi isolated (*M. anisopliae*, *Beauveria bassiana* and *Hirsutella illustris*). The results revealed that entomopathogenic fungi are an alternative way to control SHB (26, 28).

Traps; Beetle Eater Trap: It is the most effective plastic trap among the traps (Figure 4). It has a comb-shaped upper cover with 0.3 cm openings large enough for the SHB to pass through. SHB entering through this opening dies by suffocation (8, 28).

Beetle Barn Trap: This type of trap has a flat and rectangular structure. Made of black plastic, it is 9 cm long, 7.5 cm wide and 0.7 cm high. There are small openings of 1.3×0.3 cm on each side. SHB adults can pass through these openings, but not honey bees. A 2 cm^2 strip containing 10% coumaphos was placed in the middle of the trap. This trap can be put on the hive bottom board or the top of the frames (7, 8, 28).

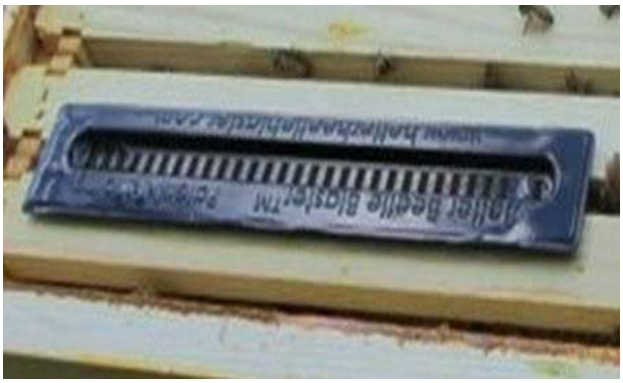

Figure 4. Beetle Eater Trap (Possum Catchers Pty Ltd.).

Figure 5. Beetle Barn Trap and Hood Trap (https://www.google.com.tr/Fbeetle-barn-reusable- beetle-hood trap).

Hood Trap: Oil or apple cider vinegar is placed in this trap made of transparent plastic, which is 15 cm long, 2.5 cm wide and 8 cm deep. The cover on it has a 12.8×0.3 cm opening so that the SHB adult can enter, but the adult bee cannot. This trap, which is attached to the bottom of an empty frame, is placed near the hatchery or on a hive floor as the first or tenth frame (8, 28).

Hive Bottom Board Trap, Full Pollen Traps: The SHB adult reaching the hive comes directly into the trap and falls into the tray with mineral oil at the bottom through the opening on the trap and disappears by suffocating (8).

What needs to be done to be protected our country?

- The purchase of all kinds of beekeeping materials and breeding stock from contaminated countries should be kept under control.
- Suspicious hives should be reported to the provincial and district directorates of agriculture, research institutions and universities.
- Manufacturers and organizations should be informed about this factor.
- Especially beekeepers' collection areas (Thrace, Muğla, etc.) should be kept under control during the season.
- The life cycle should be known in detail, and knowledge should be gained on how to recognize the larva and adult.

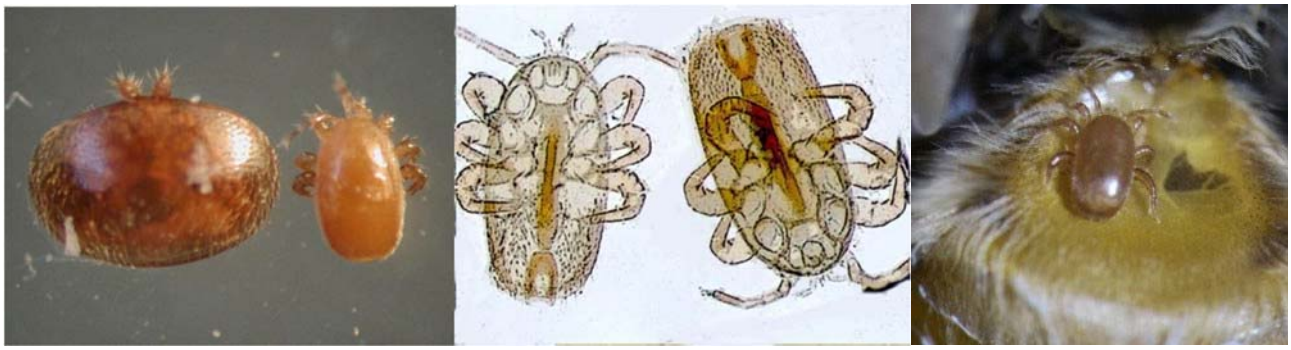

Figure 6. *Varroa* and *Tropilaelaps* spp. (ACBO), adult mite (Current in Insect Science) distributions and biology.

- Inspection of hives should be routinely performed to monitor the presence of SHB.

- In the importation of bumblebee colonies for pollination, control should be carried out in terms of SHB.

- Care should be taken in terms of the SHB factor when importing raw beeswax and honeycomb honey, as well as soil or compost planted/planted plants for commercial purposes.

- Honeycombs stored with honey or filtered should be checked regularly against the risk of SHB.

- A fluorescent light source placed on the ground at the honey extraction site will attract the larvae that seek soil to pupate. These larvae are then collected and destroyed.

- Since the SHB population develops easily in light sandy soils, apiary should not be established in these areas, it should be tried to be created on heavy clay soils (8, 10).

Tropilaelaps spp. mite

Significance of the Disease: *Tropilaelaps clareae* is a mesostigmatid parasite belonging to the Laelapidae family and is a parasite of both adult and juvenile honeybees. The agent, which is estimated to be transmitted from rat colonies, was first detected in *Apis dorsata* in the Philippines in 1961 (1-3, 9, 11, 13). After the European honeybee (*A. mellifera*) was brought to Asia, the mites infecting this species have become one of the most dangerous parasites of *A. mellifera* today. Today, although their spread is limited to the Asian continent and Kenya, they are a very serious threat to world beekeeping. Mites feeding on larvae and pupae of honeybees cause damage similar to *Varroa* infestations (15). After being observed in Iran, the mite was included in the list of notifiable diseases in our country in 2012 (9).

The place of the *Tropilaelaps* types in the system is as follows (9, 15, 29, 30);

Class: Arachnida
Underclass: Acari
Above knee: Parasitiformes Sequence: Mesostigmata
Family: Laelapidae
Genus: *Tropilaelaps*

Tropilaelaps clarae (*Apis mellifera, Apis dorsata*), *Tropilaelap mercedesae* (*Apis mellifera, Apis dorsata*), *Tropilaelaps koenigerum* (*Apis dorsata*), *Tropilaelaps thaii* (*Apis laboriosa*).

Adult mites are arthropods less than 1 mm long, dorsoventrally flattened, and longer than wide. Colors range from bright red to brown (Figure 6). It can be observed that they move quickly on honeycombs and on adult bees in the infected colonies. Their bodies are not able to divide into head, thorax, and abdomen and are covered with a hard layer of chitin (1, 9, 11, 15, 27, 30, 33).

Distributions and Biology: The natural geographic range of *Tropilaelaps* species is generally the tropical and subtropical regions of Asia where they cause very significant economic losses. The prevalence of the parasite has increased significantly in the last 50 years, although it does not have as much intercontinental spread as *Varroa destructor*. *Tropilaelaps* spp. was first seen in the Philippines in 1961, and has spread to honeybees in Vietnam, India and Afghanistan 7 years later (1, 15, 27, 29). Today, it has been detected in Afghanistan, Burma, China, Hong Kong, Java, Malaysia, Pakistan, Philippines, Taiwan, Thailand, Vietnam and Iran in Asia. It has been reported as a case report in Kenya. When *T. clareae* was above 46% when coexisting with *V. destructor* in *Apis mellifera* colonies, *V. destructor* remained below 5%. It was found more intensely in baby bee cells, showing that it reproduces faster than *Varroa* and colony destruction is more. Studies have shown that *T. clareae* infestations are faster, but they are affected more quickly by drug applications (3, 29, 33). No parasite has been found in our country in the studies carried out to date (6, 8, 36).

The development of *Tropilaelaps* spp. is very similar to the biology of *V. destructor*. As in *Varroa,* its life cycle has two periods: a) pre-reproductive (phoretic) period, b) reproductive (larval) period. In the development of the parasite, there are egg-larva-protonymph- deutonymph- and adult stages (6, 29, 30, 33).

Pathogenity and Clinical Symptoms: The pathogenicity of *Tropilaelaps* mites is similar to that of *Varroa*. As the mite larvae developing in the brood buds feed on the hemolymph of the honeybee fry (larva, pupa), they prevent them from getting the essential nutrients necessary for their development. As a result, damage and death occur in bee broods (6, 22, 29). Because the parasite is fully adapted to the young eyes, it is more dominant and disrupts the development environment of *Varroa* when co-existing with *Varroa destructor* in the same colony (6, 8, 22, 30).

- It causes rapid pup deaths and prepares the environment for secondary bacterial and viral factors. Although *Tropilaelaps* infestations are also seen in adult bees, they cannot be fed wellwith hemolymph because their mouth organelles are atrophic (6, 8).

- They exacerbate the pathogenicity by transmitting the deformed wing virus (DWV) to the honeybees (Figure 7). The most important signs are parasites in many different developmental stages when the brood eyes open, and bees without wings, deformed legs, or missing extremities in the hive exit hole. The abdomen is shortened (6, 8, 22).

- The number of hive colonies decreases rapidly. It is noteworthy that honey flow time yield islow. Especially in untreated colonies, the rapid decrease in the number of *Varroa* and as a result the extinction of the colony or the abandonment of the hive can be seen (6, 8, 30).

Diagnosis: It is done by detecting the cause of the disease. Morphologically, it should be differentiated from other ectoparasites, especially *Varroa*. The parasite moves longer and faster than *Varroa*. Approximately 100-200 adult bees are placed in powdered sugar, shaken linearly for 5 minutes, passed through 0.4 mm sieves, and the presence of the parasite can be revealed, as well as the presence of closed brood cells (6, 8). It is easier to reveal the presence of honey bees, especially in the pupal period. By placing a sticky light-colored paper on the bottom of the hive and giving smoke to the colony, parasites falling on the paper are seen (6, 8, 30).

Control: Successful results can be obtained with biological and chemical control methods.

- In the biological method, the queen bee is imprisoned in a small cell in the hive, preventing the formation of new brood cells and disrupting the breeding environment of the parasite. Removal of closed brood cells is a good option in colonies where the parasite is positive (8).

- In chemical control, fluvalinate vapor, flumethrin, coumaphos and amitraz treatment for *Varroa* treatment are used. In recent years, the use of thymol, acetic and formic acid has been tried. Positive results have been obtained from the use of formic acid for a total of 80 ml/4 weeks against larval infestation and acetic acid against adult beetles (6, 8, 27).

- The use of formic acid was found to be healthier. When the disease factor is detected, it should be reported, colonies should be strong and pollen-trapped, especially migratory beekeeping, which is an important fact of our country, should be controlled (8, 25).

- Beekeepers should be informed about this issue, as *T. clareae* is a mandatory factor. Breeding, and relocating the colony give successful results (8).

- Bee and bee products should not be bought from countries such as Iran, and colonies on the Iranian border should be pushed back 5-10 km from the border (6, 8, 33).

General Recommendations

- Establishing consulting and supporting institutions that could provide required information on standards, improved beekeeping, alternative products to honey, control of bee diseases and pests, availability of pastures, resources of nectar, etc.

- Enforcing practice of record-keeping on production and health at the apiary.

- Stimulating certified reproduction apiaries which will produce healthy reproduction material such as queen bees and colonies.

- Stimulating good bee husbandry, good hygiene practice, and apiary management.

- Further studies that will shed light on the above issues, and influence the future control programs of bee diseases have to be conducted.

- The eight basic IPM (Integrated pest control) beekeeper principles should be used when prevention is limited with protection measures against all bee diseases and pests:

a) Acceptable pest level
b) Preventive culture practices
c) Monitoring practices
d) Genetic control
e) Mechanical control
f) Physical control
g) Biologic control
h) Chemical control

- A control program starting with *Varroa* control will both be a measure to prevent other bee diseases and establish an early warning concept for diseases and pests which can be introduced later (*Tropilaelaps* spp., small hive beetle). Furthermore, the use of chemical medicines will be controlled (4, 5, 8, 17, 27, 33).

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